Contaminant Report Number: R6/216H/99
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U.S. FISH & WILDLIFE SERVICE
REGION 6

CONTAMINANTS PROGRAM

BIOASSESSMENT OF AVIAN BOTULISM AT BENTON LAKE NATIONAL WILDLIFE REFUGE, MONTANA

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INTRODUCTION

Avian botulism is a paralytic, often fatal, disease caused by the ingestion of a toxin produced by the bacterium, Clostridium botulinum. The disease occurs almost annually in Montana and in other western and Great Plains states, resulting in extensive losses of wild birds. The precise factors leading to avian botulism outbreaks are unkown, but the outbreaks are often perpetuated by a well-understood bird-maggot cycle (Jensen and Allen 1960). Toxin production follows bacterial spore germination during multiplication of the vegetative form; this initial toxin production process is poorly understood. Important environmental factors that contribute to the initiation of avian botulism include abrupt changes in water quality parameters such as dissolved oxygen, pH, and ambient temperature (Friend and Cross 1995).

Current detection of a botulism outbreak is dependent on the occurrence of dead or sick birds during field surveys (Reed and Rocke 1992). A recently developed alternative is the sentinel Mallard (Anas platyrhynchos) method (Rocke and Brand 1988), but the necessary labor and materials are too expensive for routine use by the Refuges. We proposed to examine water quality trends for possible association with the onset of botulism outbreaks to develop a procedure for an "early warning system" to aid wetland managers in dealing with botulism. We proposed to collect water quality and bioassay data before the onset of botulism outbreaks and to use those data to predict the onset of future outbeaks. Our objectives were to:

1) assess the utility of water quality measurements to predict the onset of botulism outbreaks;

2) assess the utility of Microtox bioassay procedures to detect the botulism toxin under field conditions; and

3) assess the utility of daphnia (Daphnia magna) bioassay procedures to detect the botulism toxin under field conditions.

METHODS

We selected 2 wetland units, Unit 3 and Unit 5, at Benton Lake National Wildlife Refuge (NWR), based on their recommendation by the Refuge staff as historically botulism-prone wetlands. Within each wetland, we established a sampling site in a portion of the unit with convenient access and water deep enough to permit operation of the monitoring equipment.

Water quality parameters were monitored with continuous-recording Hydrolab DataSonde 3 and Reporter/Surveyor data loggers. In 1995, data were recorded from 1 JUL to 26 SEP in Unit 3 and from 8 AUG to 26 SEP in Unit 5. In 1996, data were recorded from 15 JUL to 12 AUG in Unit 3 and from 9 JUL to 21 AUG in Unit 5. Parameters recorded included temperature, pH, specific conductance, salinity, and dissolved oxygen. From the abundant water quality data collected by the data loggers, we analyzed only those readings recorded at 1000 hrs on the days bioassay samples were collected. We used only data collected at a single time of day to control for diurnal fluctuations in some parameters (e.g., temperature, dissolved oxygen).

We collected raw water samples twice weekly from each site during the water quality monitoring period. From each 2 L sample, 1 L was reserved for Microtox and daphnia bioassays and 1 L was submitted for analysis of trace element concentrations to the Research Triangle Institute, Research Triangle Park, NC. Samples were analyzed for arsenic and selenium by hydride generation atomic absorption spectroscopy, for mercury by cold vapor atomic absorption spectroscopy, and for other trace elements by inductively coupled plasma atomic emission spectroscopy. We examined those trace element data in order to determine if they might affect the bioassay organisms. That is, mortalities among the bioassay organisms might occur due solely to the effects of increasing trace element concentrations, independent of the effects of botulism.

The Microtox suite of bioassays provides for rapid assessment of toxicity by exposing photoluminescent bacteria to sample water and then measuring the reduction in their light output (Ribo and Kaiser 1987). We conducted a Microtox 89.3% screening procedure on a portion of each water sample collected in 1995 and 1996, to screen the samples for any indication of toxicity.

We conducted static acute daphnia bioassays on a portion of each water sample collected in 1995. The method used was a variation of an established procedure described by Peltier and Weber (1985) and the American Society for Testing and Materials (1989). The methods differed from the standard procedure in that 4 replicates of 100% concentrations of sample water, rather than serial dilutions, were used in the test beakers, and pH, dissolved oxygen, and specific conductance were measured only at the beginning and end of each test. Our modifications were intended to provide larger sample sizes of undiluted samples. Spring water obtained from inflow to the Giant Springs Fish Hatchery near Great Falls, MT, was used as control treatment water. Newly hatched daphnia were obtained from the National Biological Service's Midwest Science Center, Columbia, MO.

RESULTS AND DISCUSSION

Botulism outbreaks were not detected in 1995 or 1996. Fewer than 40 bird carcasses were recovered from either unit in 1995. In 1996, 2 carcasses were recovered from Unit 3 and 18 carcasses from Unit 5. The majority of carcasses recovered were those of local hatchlings that may have died from causes other than botulism.

Water from both wetland units caused reduced light output in Microtox bioassays during late July and early August in 1995, in the apparent absence of botulism. A similar but much less pronounced pattern appeared in data collected from Unit 3 in 1996 (Table 1).

We could find no consistent association between Microtox response and concentration of any trace element sampled in 1995. Trace element concentrations were similar in Units 3 and 5 (Table 2). In samples from Unit 3, we found significant negative correlations between arsenic (Pearson's product-moment correlation coefficient, $p = 0.014$), magnesium ($p = 0.019$), and vanadium ($p = 0.021$) and Microtox light output. That is, when concentrations of those elements were high, Microtox light output was low, indicating toxicity. However, in samples from Unit 5, those same elements showed no significant correlations with Microtox light output, and all but vanadium were positive rather than negative. We suspect that there was no real effect of any particular trace element on Microtox. We could find no evidence that the increased summer concentrations of all elements (i.e., conductivity and salinity) had an effect in combination.

Equipment failures reduced the number of wetland unit-days for which we were able to obtain both water quality and bioassay data to 17 (Table 1). Water quality measures became more extreme and trace element concentrations increased as wetlands dried up in summer. Examination of the association between water quality measures and Microtox light output is a problem for time series analysis. Unfortunately, our data were insufficient for such analysis. While we had ample water quality data and they formed a true time series, our Microtox bioassays were performed at irregular intervals. A simple exploration of the data suggests that, among the parameters measured, only pH seems to vary inversely with Microtox light output, over the pH range measured. Since all pH values measured were alkaline and above the optimal pH range of test sensitivity (Ribo and Kaiser 1987), the Microtox bioassays could represent unreliable responses due to increased alkalinity.

Only 1 water sample showed any indication of toxicity to daphnia. Twelve of the 40 daphnia exposed to the sample taken from Unit 5 on 31 July 1995 died during the test, while only 1 of 39 controls died. Unfortunately, that sample was collected on a day for which we have no water quality data. Microtox data for that date showed the sample to be highly toxic. No other samples from Unit 5 appeared to be any more toxic to daphnia than was the control water. The daphnia bioassay was less sensitive to waterborne toxicants present at Units 3 and 5 than was the Microtox bioassay.

CONCLUSIONS

The absence of botulism outbreaks in both years of this study deprived us of the opportunity to evaluate the relationship of our water quality and bioassay data with the onset of botulism events. However, water samples from both wetland units were highly toxic to the Microtox organisms at some point during the study, even in the absence of a botulism outbreak, and we could detect no association between water quality or trace element concentrations and the results of Microtox bioassays. We conclude that, due to variation in the background response of the Microtox bioassay in the absence of a botulism outbreak, the Microtox bioassay has little utility in predicting the onset of botulism. Other invesigators have found the Microtox system poorly suited to assess contaminant effects in wetlands (Dieter et al. 1994).

The daphnia bioassay was not sensitive to the range of water quality values or trace element concentrations experienced in 1995. Since no botulism outbreaks occurred during this study, we do not know how predictive daphnia bioassays or water quality measures might be of an outbreak.

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Table 1. Water quality parameters and bioassay results from water samples collected from Units 3 and 5 at Benton Lake NWR. Microtox bioassay results are presented as the mean of the sample light output of 4 replicates taken 15 minutes after exposure, as a percentage of the undimished control light output. Daphnia bioassay results are presented as the number of mortalities per number exposed, for both test organisms and controls.

Date	Temperature (° C)	pH	Specific conductance (µS/cm)	Salinity (ppt)	Dissolved oxygen (mg/L)	Microtox bioassay (%)	Daphnia bioassay Test	Daphnia bioassay Control
UNIT 3								
15 JUL 95	20.0	9.1	2,760	1.5	13.00	66.25		
17 JUL 95	21.0	9.1	2,808	1.5	11.00	57.25	0/42	0/40
23 JUL 95	19.0	8.9	2,850	1.5	8.40	11.00	0/41	0/40
27 JUL 95	16.0	9.0	3,142	1.7	7.40	72.00	0/39	2/41
31 JUL 95	16.0	9.2	3,256	1.8	8.90	16.75	0/40	0/40
3 AUG 95	18.0	9.4	3,477	1.9	8.45	16.00	0/39	2/41
10 AUG 95	16.0	9.5	3,591	2.0	6.90	13.25	1/39	0/40
14 AUG 95	13.0	9.5	3,687	2.0	6.50	18.50	1/41	1/41
21 AUG 95	17.4	9.7	3,638	2.0	7.11	3.75	1/40	2/41
25 AUG 95	18.0	9.5	3,792	2.1	4.60	8.25	1/40	1/41
24 JUL 96	19.5	9.6	4,416	2.4	5.17	0.50		
1 AUG 96	19.6	9.6	5,409	3.0	0.13	89.00		
8 AUG 96	14.0	9.9	6,282	3.5	0.44	0		
12 AUG 96	15.9	9.0	6,981	3.9	0.48	0.25		
UNIT 5								
10 AUG 95	13.8	10.2	5,268	2.9	7.94	0	0/37	0/43
21 AUG 95	16.0	9.6	6,812	3.8	7.30	5.00	0/38	0/40
25 AUG 95	14.3	8.7	7,417	4.1	4.12	51.33	0/39	1/39

collected from Benton Lake NWR. Data are presented as geometric means with ranges in parentheses.

Element	Unit 3 (n = 12)	Unit 5 (n = 11)
Aluminum	0.187 (0.036-0.937)	0.239 (0.067-8.600)
Arsenic	0.034 (0.017-0.042)	0.010 (<0.006-0.058)
Barium	0.054 (0.030-0.067)	0.054 (0.038-0.157)
Beryllium	NC[A] (<0.001-<0.001)	NC (<0.001-<0.001)
Boron	0.282 (0.255-0.316)	0.165 (0.120-0.268)
Cadmium	NC (<0.001-0.004)	NC (<0.001-0.005)
Chromium	NC (<0.006-<0.006)	NC (<0.006-0.011)
Copper	0.006 (<0.006-0.012)	0.007 (<0.006-0.021)
Iron	0.095 (<0.022-1.030)	0.115 (<0.022-9.113)
Lead	NC (<0.006-<0.006)	NC (<0.006-0.011)
Magnesium	225.2 (188.3-286.4)	273.3 (190.1-469.0)
Manganese	0.020 (0.002-0.117)	NC (<0.002-0.226)
Mercury	NC (<0.001-<0.001)	NC (<0.001-<0.001)
Molybdenum	NC (<0.004-0.009)	0.011 (0.006-0.022)
Nickel	NC (<0.006-0.006)	NC (<0.006-0.021)
Selenium	NC (<0.006-<0.006)	NC (<0.006-<0.006)
Strontium	0.879 (0.694-1.048)	1.125 (0.927-1.848)
Vanadium	0.009 (<0.004-0.019)	0.021 (0.010-0.057)
Zinc	NC (<0.011-0.018)	NC (<0.011-0.039)

[A] NC = Not calculable.